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PHYSICAL CHARACTERISTIC AND VIABILITY OF *LACTOBACILLUS ACIDOPHILLUS* MICROPARTICLE USING HPMC K100LV AND HPMC K4M AS MATRICES

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ABSTRACT

Objective: *Lactobacillus Acidophillus* is widely used in food supplement that requires viability in the range of 10^6 – 10^{12} cfu /per gram. This microbe is not stable in acidic conditions and has been reported that the number of their colonies in fermented milk products decreased by 5 logs in acidic solution. In the other side,, the probiotic microbe is required to survive GI tract passage and remains viable. In order to maintain their viability and increase their stability in such environments, *Lactobacillus Acidophillus,* were entrapped in HPMC K100LV as a protectant using microencapsulation technique.

Methods: The *Lactobacillus acidophillus* cells were inoculated in MRS broth media at 37 ^{0}C for 48 hours. A number of cell (more than 10^9 cfu/ml) wassuspended in 10% milk solution. The cultures were then cooled at 20^{0}C for about 12 hours, and subsequently mixed with suspension of HPMC K4M 0%, 0,3%, 0,5%, 0,8% and HPMC K100LV 0%, 0,5%, 1% and 1,5%. The mixtures were then dried using spray drying method.. The Physical characteristics of the microparticles and the viability of the microbe in the microcapsules were evaluated, including the microparticle morphology, size and moisture content. The viability was measured by Total Plate Count method.

Results: showed that all microparticles exhibited spherical shape with the size in range of 6,0 – 8,0 um. The moisture content was in range of 8,0 – 10,0 %. The highest viability was obtained by formulation of HPMC K100LV 1% and HPMC K4M 0,3%.

Conclusion: of this study is that HPMC K100LV and HPMC K100M are good matrices for probiotic microcapsule based on the physical characteristic and the microbe viability (probiotic viability > 10^7 cfu/g)

Keywords: *Lactobacillus acidophillus, HPMC K100LV, HPMC K4M, Physical characteristic, Viability*

INTRODUCTION

Most of the comensal bacteria, which are more than 2000 species live in human intestinal area and provides health benefits to the host, because of the improved microbial balance in the intestine. They are from two procaryotic microorganisms, i.e. *lactobacillus* and *bifidobacterium* with th various different species. The intestinal microbial colonization starts at birth and continues during the subsequent phases of life that form an individual intestinal microbiota. Such microorganisms are known as probiotics [1,2,3,4]. It has been generally accepted that the probiotics exerts health benefits if their minimum count is about 10^6 cfu per g of food per consumption (5). They should survive GI tract passage and adhere to the intestinal mucosa or other target sites in GIT [4]. It has been known that there were significant reduction of the number of living microorganisms in GIT. The reduction process in gastric juice was a pH dependent where the pH of gastric juice is ranging from 1 to 4. It was reported that pH plays an important role in cells death. There were 3,5 and 2,2 log reduction on e viability after *lactobacillus acidophillus* were inoculated for 90' in simulated gastric juice at pH and 2,5 respectively [6,7]. Another research reported that *lactobacillus* and *bifidobacterium* sp. lost more than 90% after have been exposed to simulated gastric juice at pH 2 [6,7,8]

Lactobacillus acidophillus is widely ed regarding to the health benefiting effect. Lactobacillus are extensively incorporated into yoghurts, cultured milk drinks, cheese or as dietary supplements in the form of dried dosage forms. [10,11]. Entrapment of living cells in a matrix called microencapsulation is a method used for protection of the immobilized materials as well as for a controlled release in intestinal mucosa. The outer layer or the wall of the microcapsule will protect the cell against moisture, heat, strong acidic and other extreme conditions during storage, manufacturing as well as during digestion Microencapsulation method has a lot of advantages, including the protection effect from misture, heath or other extreme conditions. However, it still causing significant cell death, since most common encapsulation method uses spray-drying that involves heating [11,12,13]. The microencapsulation method, allow the incorporation of *Lactobacillus acidophillus* and another probiotic microorganism. in which a polymer acts as outer layer or protectant [12,13]. A number of microencapsulation techniques including: spray drying, inclusion complication, extrusion, co-crystallization and gel entrapment (extrusion, emulsification, coacervation). Spray-drying (dehydration method) has been oftenly used in industries among other cell preservation methods, because it is cheaper, cost effective and suitable for a large-scale production. The cost effectiveness spray drying was estimated to be six time cheaper per kilogram compared to freeze drying [14]. T disadvantage of this method is that many microorganisms can not tolerate the drying process due to the high heat involved. Other factors that may affect the survival during spray-drying process are the type of the strain, growth phase, protective medium used, outlet temperature of spray-drier and pre- treatment of the culture. [13,14] This study investigated the use of HPMC K100LV (0%, 0,5%, 1% and 1,5%) and HPMC K4M (0%, 0,3%, 0,5% and 0,8%) as protectant. The sed of HPMC to enhance probiotic surival through heat treatment s been studied previously. HPMC is safe, nonionic polymer that minimize interaction problems when they are used in acidic, basic, or other electrolytic stem. They can be used for preparing formulations with water soluble or insoluble drugs and at high or low doses. [15]. The polymer is therefore useful for the cell because it assist the adaption of microbe to the environment. Furthermore the polymer reduces the osmotic differences een the cellular internal compartment and the environment. In this study, a dried probiotic powder was produced using spray drying methods with perature of 60^0 C.

MATERIALS AND METHODS

Materials

Lactobacillus acidophillus was obtained from Microbioliogy Laboratorium-Brawidjaja University, Indonesia.

HPMC K4 M and HPMC K100LV (Pharmaceutical Grade) were purchased from PT. Lawzim Zecha. *de Man Ragosa Shorpe* (MRS) broth media was purchased from

Methods

1. Preparation of Spray Dried Milk-Probiotic –HPMC K4M

The *Lactobacillus acidophillus* cell in amount of 1 ose were inoculated in MRS broth media at 37 ^{0}C for 18 hours. The number of cell should be more than 10^9 cfu/ml and suspended in 10% milk solution.

The cultures were than cooled at 20^0 C for about 12 hours, and subsequently mixed with suspension of HPMC K4M 0%, 0,3%, 0,5%, 0,8% and HPMC K100LV 0%, 0,5%, 1% and 1,5%.,

The mixtures were then dried using spray dryer (Lab-Plant SD-Basic Spray Dryer)

Physical Characterizations

1. pH measurement

The pH of the milk and milk-probiotic was measured using a pH meter SCHOTT glass mainz, CG 842 type.

Table 1: Composition of Probiotic Microparticle with HPMC K4M and PMC K100LV as a matrix

Composition	HPMC K100LV				HPMC K4M			
	F1	F2	F3	F4	F5	F6	F7	F8
Milk-Probiotic	400 ml	400 ml	400 ml	400 ml	400 ml	400 ml	400 ml	400 ml
HPMC K100LV	-	2,5 g	5 g	7,5 g	-	-	-	-
HPMC K100M	-	-	-	-	-	0,15 g	0,25 g	0.40 g
Aquadest	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml

2. Viscosity Measurement

The viscosity of the milk and milk probiotic was measured using VT-04 viscometer

3. Moisture content determination

The moisture content of the microparticles was determined using Moisture Analyzer HB43-S Metler Toledo

4. Size and morphology of probiotic microparticles

The size of microparticles was measured using optical microscope. The morphology of the particles was visualized using SEM (FEI-type:inspect-S50)

Viability measurement

Viability of lactobacili in milk (before spray drying) and in microparticle (after spray drying) was assesed using MRS media. 1 ml probiotic milk or 1 gram microparticel was mixed with 9 ml sterile Phosphate Buffer Salin (PBS) solution. A serial dilution (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9} and 10^{-10}) of this suspension was made and then spread on the MARS agar. and incubated at 37 oC for 48 hours. The viability of the probiotic was reported as TPC (cfu/ml or cfu/g) and survivability (N/No x 100%).

Statistical analysis

One Way ANOVA with Honestly Significant Differenc (HSD) Tukey was used to analyze the results. Confidence limits of 95% ($\alpha = 0,05$) were used to determine statistical significance.

RESULT AND DISCUSSION

Table 2: The pH and viscosity of milk and milk-probiotic after 24 hours

Material	pH	Viscosity
Milk	6.41 ±0,01	0,4±0,01
Milk-Probiotic	4,33±0,00	0,7±0,00

The results showed that the fermented milk has a lower pH compared to the non-fermented milk ($p<0.05$) (Table 2). The decrease of pH indicated that there were accumulation of lactic acid and acetic acid as a result of lactosa metabolism. *Lactobacillus acidophillus* has an ability to metabolize lactose to lactic acid, acetic acid and CO2. The viscocity data showed that milk- probiotic had a greater viscosity than normal milk. It indicated that microorganisms in the milk contribute the solid component and resulted in increasing viscosity. Spray dried powders of lactobacillus acidophillus -containing microparticle with HPMC has a mean size higher than microparticle without HPMC, and increasing HPMC concentration resulting in increasing particle mean size.

Table 3: The Mean Particle size and moisture content measurement

Measurement	F 1	F II	F III	F IV	F V	F VI	F VII	F VII
Mean Diameter (µm)	6,725	7,283	7,142	8,492	7,65	7,39	8,11	8,07
Moisture Content (%)	10,54	7,41	7,79	8,70	10,45	10,09	9,51	8,96

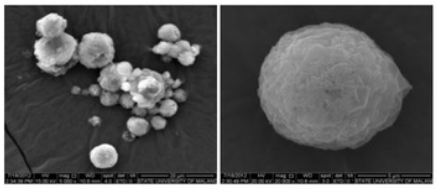

Fig. 1: Morphology of Probiotic Microparticle without a HPMC (5000x and 20.000x)

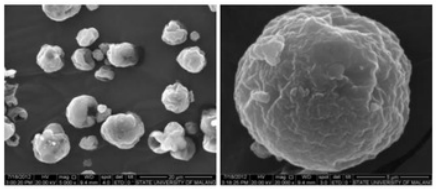

Fig. 2: Morphology of Probiotic Microparticle with HPMC K100LV (5000x and 20.000x)

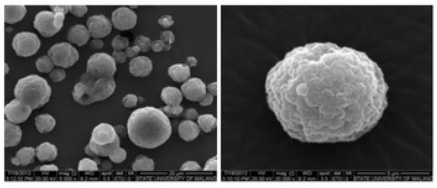

Fig. 3: Morphology of Probiotic Microparticle with HPMC K4M (5000x and 20.000x)

It might be explained that increasing HPMC concentration will increase the composition of wall microparticle. The microparticle morphology is spherical with rough surface. It might be explained

that rate of evaporation was not proportional to the rate of film formation.

The data obtained in these study showed that all microparticle had moisture content greater than recommended range, i.e. 2,80% – 5,60% [13] and the moisture contents increased with the greater matrix concentration (Table 3). This can be explained that the matrix used (milk and HPMC) exhibited a higroscopic properties. The drying temperatur, 60 ^{0}C was too low and was not necessary in regards to fulfill the recommended moisture content.

The morphology of microparticle (SEM image, Fig. 1) showed that the mcroparticles was spherical and have a rough surface.

Table 4: TPC value (cfu/ml or cfu/g) of *Lactobacillus acidophilus* in the probiotic microparticle with HPMC K100LV and HPMC K4M as a matrix

Group	HPMC K100LV	HPMC K4M
Probiotic milk	$306,3x10^7 \pm 10,9x10^7$	$162 \times 10^{12} \pm 9,5 \times 10^{12}$
Formula I	$5,7x10^7 \pm 1,5x10^7$	$157 \times 10^{10} \pm 5,29 \times 10^{10}$
Formula II	$4,8x10^7 \pm 3,2x10^7$	$295 \times 10^{10} \pm 1,53 \times 10^{10}$
Formula III	$24,4x10^7 \pm 6,3x10^7$	$139 \times 10^9 \pm 2,53 \times 10^9$
Formula IV	$20,5x10^7 \pm 5,1x10^7$	$3,30 \times 10^7 \pm 2,64 \times 10^7$

Table 5: Viability (%)of *Lactobacillus* in the probiotic mikroparticle with HPMC K100LV and HPMC K4M as a matrix

Group	HPMC K100LV	HPMC K4M
Probiotic milk	100,00 ± 0,00	100,00 ± 0,00
Formula I	81,69 ± 1,29	85,99 ± 0,074
Formula II	81,03 ± 0,21	87,93 ± 0,153
Formula III	88,34 ± 1,01	78,58 ± 0,125
Formula IV	87,54 ± 1,01	53,01 ± 0,285

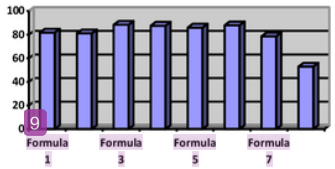

Fig. 4: Viability (%) of *lactobacillus acidophillus* in the microparticle using HPMC K100LV and HPMC K4M as a matrix

Table 4 and 5 showed the viability (cfu) and percentage survival (%) of *lactobacillus acdophillus* probiotic. The data indicated that viability of microparticles was lower than probiotic milk (before spray dried). There was a viability reduction by approximately 20%. The viability reduction of microparticle compared with probiotic milk can be explained as a result of heating process during spray drying.

No significant difference (p> 0,05) were obtained between *Lactobacillus Acidophillus* viability of HPMC K100LV 0% and 0,5%, HPMC K4M 0% and 0,3%. It showed that both polymers have an equal protective effect when using HPMC K100LV 0,5% and HPMC K4M. The profile of *lactobacillus acidophillus* viability in HPMC K100LV group was different from HPMC K4M group (Statistic?). In HPMC K4M group, the greater the concentration, the greater the viability. It can be explained: that increasing HPMC concentration will result in increased viscosity and increasing microbe entrapment. During the viability test process, part of the cell still entrapped. However, it was still higher than 10^7 cfu/g ($3,30x10^7$ cfu/g)

CONCLUSION

1. *LactobacillusAcidophillus* microparticle using HPMC K100LV and K4M as a matrices has a spheric and rough, and the particle size ranging from 6,0 to 8,0 µm.
2. *LactobacillusAcidophillus* microparticle using HPMC K100LV and K4M as a matrices has moisture content 7,0 – 10,0 %, out of recommended range, 2,80 – 5,60
3. *Lactobacillus Acidophillus* microparticle using HPMC K100LV and K4M as matrices, result a probiotic viability > 10^7 cfu/g

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